

## MRSA in herds of fattening pigs in Germany – Associated risk factors

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### Abstract

We investigated the association of putative risk factors with the prevalence of MRSA in herds of fattening pigs. Dust samples of 282 herds in Germany were collected in 2008. Information regarding herd characteristics and management practices was collected in a standardized questionnaire.

Dust samples were pooled per farm and cultured using selective enrichment and chromogenic media. Presumptive MRSA-isolates were confirmed by multiplex-PCR for the detection of 16S rDNA, *nuc* and *mecA* genes. The association of management factors with prevalence was determined using univariate logistic regression. Multivariate models were not used on account of substantial multicollinearity between the variables.

MRSA were identified in 145 herds (51.4%). Using univariate analysis, large farms (>999 animals) were at higher risk of harbouring MRSA than small farms (<500 pigs, OR 4.0, 2.2-7.4). Grower- and weaner-to-finish operations were more likely to be positive compared to farrow-to-finish farms (OR 3.4, 1.8-6.3). Most farms (59%) used an all in/all out strategy with disinfection. However, these farms were at higher risk of being positive for MRSA than those without all in/all out management (OR 2.1, 1.2 -3.5). Buying weaners and growers from more than two sources increased the likelihood of being positive over raising homebred piglets (OR 4.2, 1.9-9.4).

Administration of antibiotics at group level in the past four months increased the risk for the herd to test positive compared to herds that did not report such treatments (OR 1.9, 1.2-3.1). Occurrence of clinical disease in the herd was not associated with a greater risk of testing positive for MRSA.

Results of the study confirm the hypothesis that trade and use of antimicrobials may support the spread of MRSA. The use of all in/all out management did not effectively control MRSA. However, the complex interaction between the variables requires further investigations.

### Introduction

Methicillin resistant *Staphylococcus aureus* (MRSA) have been detected in a number of farm animal species during the last years (Tenhagen et al. 2009). Pigs have been reported to harbour MRSA on all stages along the production chain and a number of reports have speculated on potential risk factors for the detection of MRSA in herds of pigs (van Duinkerken et al. 2008). The European Union has carried out a survey in 2008 to determine the prevalence of MRSA in herds of breeding pigs (EU 2008).

Exposure of consumers to breeding pigs, however, is rare. People involved in pig farming, i.e. farmers, vets and associated professions, have been shown to be at a high risk of getting colonized with livestock associated MRSA (Meemken et al. 2008). A high proportion of pigs at slaughter carry MRSA and a very high proportion of slaughter batches was positive for MRSA, i.e. contained at least one positive animal (de Neeling et al. 2007; Tenhagen et al. 2009).

It was the purpose of this study to determine, whether high MRSA-prevalences observed in slaughter pigs would also be reflected in the fattening pig herds. Furthermore, we aimed at identifying potential risk factors for the prevalence of MRSA in the herds.

### Material and Methods

Dust samples were collected in 282 fattening pig herds in 7 Federal States in Germany. Herds were chosen randomly per federal state according to a sampling plan. Allocation of herds to be sampled was according to the number of fattening pigs in herds with more than 100 animals in the respective Federal State. Dust samples were collected by official veterinarians or under their supervision. Per herd, 5 dust samples from different locations in the stable were collected and pooled for laboratory analysis. The sampling procedure

was in accordance with the methods prescribed for the EU-wide survey in breeding pigs (EU 2008). In brief, dust was collected using swabs from five locations from a surface of 500cm<sup>2</sup> each and transported to the laboratory in a sterile plastic bag.

In the laboratory, all five swabs were pooled and transferred to 100 ml Mueller-Hinton-Broth with 6.5 % NaCl and incubated for 16-20 hrs. at 37°. 1 ml of broth was added to 9 ml Trypton-Soy-Broth with 3.5mg/l of cefoxitin and 75 mg/l aztreonam. After incubation at 37°C for 16-20 hrs one loop full of broth was plated on chromogenic agar and incubated at 37°C for 24 hrs. Presumptive MRSA were subcultured on blood agar and confirmed as MRSA using the triplex PCR as described by (Poulsen et al. 2003).

Information on management aspects of the pig herd was collected using a standardized questionnaire. The following items were requested: type of farm (farrow to finish, weaner to finish, grower to finish), size of the herd (<500, 500 to 999, >999), location of the herd (Federal States), origin of the pigs (homebred, purchase from 1-2 origins, purchase from >2 origins, purchase from weaner to grower operation), use of antimicrobials at group level in the fattening period, occurrence of clinical disease in the fattening period, use of an all in / all out policy (no, yes with cleaning, yes with disinfection).

Data were compiled in a SPSS database. After crosstabulation variables were investigated with respect to their association with the outcome of the detection of MRSA in the samples using univariate logistic regression. Testing of the variables for independence revealed that independence was not given. Therefore, no multivariate model was calculated but relationships were characterized by crosstabulating variables.

## Results

Of the 282 herds included in the study 145 (51.4 %) were positive for MRSA. Table 1 displays numerical data concerning the association of risk factors with the detection of MRSA in fattening pig herds using univariate analysis.

No difference was found between herds that reported the occurrence of clinical disease during the fattening period and those that did not. Respiratory (44.7 % of herds) and gastrointestinal diseases (21.6 % of herds) were most often observed.

## Discussion

Results of our study support the hypothesis that MRSA is widespread in the fattening herds in Germany. The Federal States that participated in the study represent quite different agricultural structures, covering most of the typical situations in Germany. Even though differences in the prevalence were observed between participating Federal States, these did not seem to be related to the regional structure of agriculture. The Federal States with the highest and with the lowest prevalence were located next to each other without substantial differences in the structure of the pig industry.

Farrow to finish farms were less often positive for MRSA than weaner and grower to finish operations. In line with this, farms that did not purchase pigs were less often positive than those buying pigs from 1-2 or even more sources. All five farms buying pigs from weaner to grower operations were positive. It has been speculated, that MRSA are spread between farms via trade of colonized animals (van Duijkeren et al. 2008). Buying pigs from 2 or more sources increases the probability that positive pigs are introduced.

Table 1: Proportion of positive herds stratified by major variables and odds ratios compared to reference categories.

Parameter	Categories	No. of herds included	No. of positive herds	OR to reference category
Herd size	<500	82	24	29.3
	500 to 999	83	48	57.8
	>999	112	70	62.5
Type of operation	Farrow to finish	63	18	28.6
	Weaner to finish	36	22	61.1
	Grower to finish	181	104	57.7
Number of origins for pigs	No purchase	71	24	33.8
	1-2 origins	159	86	54.1
	>2 origins	44	30	68.2
	From weaner to grower operation	5	5	100.0
Use of antimicrobials	Yes	157	92	58.6
	No	125	53	42.4
All in / all out policy	No	90	36	40.0
	Yes, with cleaning	22	10	45.5
	Yes, with cleaning and disinfection	166	96	57.8

Farm size was associated with prevalence. This difference was pronounced when small farms were compared to bigger farms, while the two upper categories did not differ very much from each other. This can be attributed to two effects. Small farms were more frequently farrow to finish operations and did not buy weaners or growers. Furthermore, in larger units more animals are introduced and there are potentially more shedding animals and more animals that are susceptible. Hence the conditions to spread and multiply in the herd, once the pathogen is introduced are more favourable in larger pig herds.

Use of antimicrobials was also positively associated with the detection of MRSA. It is well documented that selection pressure optimizes the conditions for resistant pathogens to multiply. Once the pathogen is introduced into the herd, the use of antimicrobials may support the spread within the herd. Moreover, tetracycline and amoxicillin were the predominant group of substances used in the herds. MRSA are by definition resistant against amoxicillin. Livestock associated MRSA have been shown to be resistant against tetracyclines (de Neeling et al. 2007; Tenhagen et al. 2008).

Use of antimicrobials was also associated with herd size. The bigger the herds were, the higher the proportion of herds where antimicrobials had been used during the fattening period. However, in each category of herd size, the prevalence of MRSA was higher in herds where antimicrobials had been used compared to those without use of antimicrobials.

The implementation of an all in / all out strategy was not associated with a lower prevalence of MRSA in the herds. Moreover, the highest prevalence was observed for herd reporting an all in / all out strategy with cleaning and disinfection. This may be related to the fact that a higher proportion of these farms bought pigs from more than two sources at a time and that herds using an all in / all out policy had the highest rate of antimicrobial usage. Furthermore, *S. aureus* has been described to be fairly resistant to cleaning procedures and to have a tendency to form biofilms (Kusumaningrum et al. 2003).

In conclusion, buying pigs from various origins or weaner to grower operations and use of antimicrobials may be relevant factors contributing to the likelihood of MRSA in fattening pig herds. Seventy-nine percent (27/34) of the herds combining these two risk factors were positive for MRSA.

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